

## TNO's capabilities in immunomodulation Drug validation in *in vitro* and *in vivo* models of immunomodulation

It is well established that most, if not all, chronic inflammatory disorders are mediated or regulated by humoral and cellular components of the immune system. Many of these mechanisms represent potential targets to the treatment of immunological diseases.

TNO offers a broad range of services ranging from *in vitro* models to humanized *in vivo* models that can support the development of your lead compound. Many important targets are represented in these models. Several of these models are useful to analyze biologicals with specificity for human targets.



### ***In vitro* models**

#### *T cells and dendritic cells*

Autoreactive T cells represent therapeutic targets in autoimmune diseases. A delicate balance between effector T cells and regulatory T cells determines the outcome in the pathogenesis of chronic inflammatory disorders. However, dendritic cells are considered the master regulators of T cell differentiation. All these cell types represent potential therapeutic targets in autoimmune disease. Cultures of human peripheral blood mononuclear cells or co-cultures of T cells and dendritic cells help us identify compounds with immunomodulatory potential. Substances can for instance be evaluated based on their ability to modulate the expression of cytokines.

#### *Lymphocytes and synoviocytes*

(Co-)cultures of peripheral blood mononuclear cells, synoviocytes and cartilage are available to mimic processes involved in rheumatoid arthritis. Access to patient material allows us to identify deranged mechanisms, such as the cytokine-driven expression of metalloproteinases or cartilage damage, and to study how these mechanisms are modulated by a wide range of compounds.

### ***In vivo* models**

TNO offers a portfolio of models for chronic inflammatory diseases. This portfolio comprises models for multiple sclerosis, rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, psoriasis, contact hypersensitivity, atopic dermatitis and skin transplantation.

### Models of multiple sclerosis and rheumatoid arthritis

The multiple sclerosis model, experimental auto-immune encephalomyelitis (EAE) can be performed in various mouse or rat strains, representing different aspects of MS. EAE models are used worldwide for proof-of-concept if novel targets in autoimmunity have been identified. Different mechanisms found to play a role in autoimmunity were discovered in this model and have successfully been validated with candidate drugs ranging from SMW compounds to cytokines and antibodies specific for cell-adhesion molecules and chemokine receptors. TNO has a strong scientific track record with respect to MS and EAE. Models of choice are usually EAE in SJL mice and EAE in DA rats.

Likewise, experimental arthritis models are available in DBA mice and LEW rats to study anti-inflammatory, immunomodulatory or anti-resorptive compounds. In addition to these models of inflammatory joint disease, complementary models of joint degeneration (osteoarthritis) are available in rats, guinea pigs and dogs.

### Models of inflammatory bowel disease

TNO is developing and improving models of colon inflammation. TNO currently offers acute and chronic models of TNBS-induced colitis. A chronic model of IBD is being developed in IL-10 knock-out mice as well. Immunomodulation in these models is studied in conjunction with evaluating the anti-inflammatory potential of probiotics.

### Models of skin inflammation

A humanized mouse model of psoriasis has been established employing non-lesional skin transplanted onto immunodeficient mice. A psoriatic process is initiated by injecting autologous activated T cells into the graft. The advantage of this model is that many human targets are available. Several biologicals have been tested with success in this model. A model based on transplanted lesional skin allows for the evaluation of candidate drugs in a "therapeutic" setting.

TNO has also developed a model of atopic dermatitis based on transgenic expression of human APOC1. This model differs from the previously mentioned inflammation models, since this disease is Th2 mediated and different mechanisms are involved. It is also useful as a model of pruritus.

A rat allogeneic skin transplantation model is available to study the efficacy of immunosuppressive drugs.

Oxazolone-induced delayed-type hypersensitivity complements our skin portfolio as a model of contact hypersensitivity with short duration.

### TNO's role in identifying novel anti-inflammatory drugs

- To demonstrate efficacy *in vitro* using human blood cells or isolated subsets (e.g. dendritic cells). Read-outs: dedicated analysis (e.g. TNF- $\alpha$  production, cell activation by flowcytometry) or gene expression profiling with the use of arrays.
- To show efficacy in well-established *in vivo* models. Read-outs: symptoms associated with clinical disease.
- To gain insight into underlying mechanisms, e.g. induction of mechanisms of tolerance, employing flowcytometry, immunohistochemistry, cytokine production by lymphocytes and dendritic cells, and the production of antibodies. For inflammatory arthritis and osteoarthritis TNO can also evaluate the process of tissue degeneration by examining the products of cartilage degradation in serum and urine.
- TNO has a broad expertise in toxicity assessment and pharmacokinetics.

### Evaluation of biologicals

A new generation of drugs comprising (humanized) antibodies, fusion proteins, cytokines and growth factors may be ineffective in classical models of inflammation and require humanized models to show their efficacy. TNO has successfully developed a humanized model of psoriasis which was demonstrated to be sensitive to various biologicals. TNO aims to develop similar models for other diseases in the near future.

## TNO Quality of Life

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